NADPH Assay: Protocol for Measurement of NAPDH in Bacterial Cultures with fluorescence

Goal: To check the extracellular concentrations of NADPH.

Protocol:

Preparation:

1. **The night before:** prepare starters for the desired strains in LB (5 ml LB with 5µl antibiotics) and place in shaker overnight at 37°C

2. Dilute the starters at a ratio of 1:100 in LB, add appropriate antibiotics, and check O.D until it reaches OD600=0.6.

3. Centrifuge 5 min, 5000 rpm and discard supernatant. Re-suspend pellet in 5%LB in BA and add antibiotics and inducer if needed. Move supernatant into Erlenmeyers.

4. Each measurement time remove 1 ml of sample into Eppendorf.
    a. take 200µl in duplicate unto appropriate well and read O.D. in 600nm
    b. Centrifuge the rest on maximum speed for 2 minutes.
    c. Remove 200 µl in duplicate of the supernatant, to a 96 well plate.

5. Read samples immediately in the plate reader (Tecan-infinite 200Pro) at:
    a. Excitation 340nm
    b. Emission 465nm
    c. O.D 600nm

Results analysis:

1. Subtract blanks (fluorescence and OD) from samples readings.

2. Calculate the fraction of $\frac{Flourescence}{OD}$ for normalized fluorescence.

Written by Roni